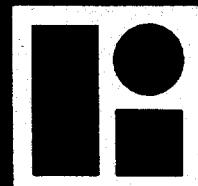


Mutagenic Evaluation of Compound FDA 75-91
Final report

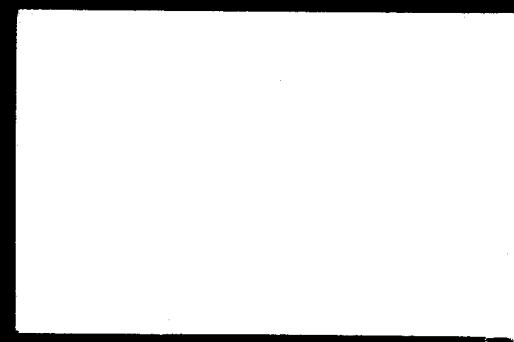
9/77
(Aluminum Potassium Sulfate)

15



Litton

BIONETICS



5516 Nicholson Lane
Kensington, Maryland
20795

MUTAGENICITY EVALUATION
OF
FDA 75-91
ALUMINUM POTASSIUM SULFATE
FINAL REPORT

SUBMITTED TO
GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY
BUREAU OF FOODS
U.S. FOOD AND DRUG ADMINISTRATION
200 C STREET, S.W., ROOM 1066
WASHINGTON, D.C.

SUBMITTED BY
LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795
LBI PROJECT NO. 2672
SEPTEMBER 1977



BIONETICS

TABLE OF CONTENTS

	Page No.
EVALUATION SUMMARY.....	1
I. <u>OBJECTIVE</u>	2
II. <u>MATERIALS</u>	2
A. Test Compound.....	2
B. Indicator Microorganisms.....	2
C. Reaction Mixture.....	2
D. Tissue Homogenates and Supernatants.....	3
E. Positive Control Compounds.....	3
III. <u>METHODS</u>	3
A. Toxicity.....	3
B. Plate Tests.....	4
C. Suspension Tests.....	4
D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions.....	5
E. Data Recording and Reporting.....	5
IV. <u>RESULTS SECTION</u>	
A. Solubility Properties of the Test Compound.....	6
B. Toxicity and Dosage Determinations for the Test Compound.....	6
C. Plate Assay Results.....	7
D. Suspension Assay Results.....	7
V. <u>INTERPRETATION OF RESULTS AND CONCLUSIONS</u>	15
VI. <u>EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS</u>	16
VII. <u>EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS</u>	17
APPENDIX - Tabulation of Data.....	A-1



BIONETICS

EVALUATION SUMMARY

The test compound, FDA 75-91, Aluminum Potassium Sulfate, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: July, 1977

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound: FDA 75-91, Aluminum Potassium Sulfate

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: December 29, 1976

2. Description: White crystals

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: Saccharomyces cerevisiae, strain D4

Bacteria Strains: Salmonella typhimurium, strains TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	4 μ moles
2. Glucose-6-phosphate	5 μ moles
3. Sodium phosphate (dibasic)	100 μ moles
4. $MgCl_2$	8 μ moles
5. KCl	33 μ moles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

<u>Assay</u>	<u>Chemical^a</u>	<u>Solvent</u>	<u>Probable Mutagenic Specificity</u>
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS ^b
	Ethylmethanesulfonate	Water or saline	BPS ^b
	2-Nitrofluorene	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard	Water or saline	FS ^b
Activation	Dimethylnitrosamine	Water or saline	BPS ^b
	2-Acetylaminofluorene	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline	Dimethylsulfoxide ^c	FS ^b
	2-Aminoanthracene	Dimethylsulfoxide ^c	BPS ^b

^a Concentrations given in the Results Section

^b BPS = base-pair substitution; FS = frameshift

^c Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.



BIONETICS
Litton

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.



BIONETICS

Litton

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.



BIONETICS

Litton

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: FDA 75-91, Aluminum Potassium Sulfate.
2. Test solvent: * Saline
3. Solubility of the test compound under treatment conditions: Soluble
4. Additional comments: White powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: February 21, 1977
2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

<u>Test Doses</u>	<u>Percent Concentration</u>	
	<u>Bacteria</u>	<u>Yeast</u>
1/4 50% Survival	0.5	1.2500
1/2 50% Survival	1.0	2.5000
50% Survival	2.0	5.0000

*The concentration of solvent was equal to the highest volume of test material added.



BIONETICS
Litton

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.



BIONETICS

Litton

SUMMARY OF IESI RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 007784249
 B. TEST DATE: MARCH 2, 1977

IESI	SPECIES	ISSUE	PLATE IESI		PLATE IESI		PLATE IESI	
			1	2	1	2	1	2
1. NON-ACTIVATION								
SOLVENT CONTROL*			30	34	19	14	21	16
POSITIVE CONTROL**			>1000	>1000	>1000	>1000	>1000	>1000
TEST	2.00000 %		53	40	16	17	11	15
	1.00000 %		---	---	13	19	19	19
	0.50000 %		---	60	48	13	19	13
			42	67	10	18	12	19
2. ACTIVATION								
SOLVENT CONTROL*			HOUSE	LIVER	29	33	27	26
	RAT	LIVER	37	36	34	23	14	23
	MONKEY	LIVER	35	28	26	34	15	11
	HOUSE	LIVER	117	536	180	154	409	487
	RAT	LIVER	99	220	212	185	450	449
	MONKEY	LIVER	669	626	162	189	434	410
	HOUSE	LIVER	27	26	20	13	21	24
	RAT	LIVER	27	26	21	18	16	12
	MONKEY	LIVER	25	30	25	17	17	11
	HOUSE	LIVER	25	30	25	17	17	11
	RAT	LIVER	26	17	18	21	19	24
	RAT	LIVER	14	14	19	18	12	21
	RAT	LIVER	28	22	19	20	21	18
	MONKEY	LIVER	29	31	34	31	29	31
	MONKEY	LIVER	29	26	20	20	24	27
	MONKEY	LIVER	16	21	21	20	22	21

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE *** TA-1535 ANTH 100 UG/PLATE
 TA-1537 QM 20 UG/PLATE *** TA-1537 AMQ 100 UG/PLATE
 TA-1538 NF 100 UG/PLATE *** TA-1538 AAF 100 UG/PLATE
 TA-98 NF 100 UG/PLATE *** TA-98 AAF 100 UG/PLATE
 TA-100 MNNG 2 UG/PLATE *** TA-100 ANTH 100 UG/PLATE
 NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

- INDICATES NO DATA WAS TAKEN.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXH34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

NONACTIVATION COMPOUND 007784249

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA98 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5	CONTROLS
NAN		338.20	12.73	14.11	11.85	9.80	16.81	13.67
NAP		580.47	142.32	99.71	120.00	195.21	216.15	189.62
NA1		196.13	17.14	7.66	3.96	9.93	19.14	5.20
NA2		186.35	16.44	9.20	2.49	7.67	8.99	8.17
NA3		205.41	14.84	11.11	5.92	5.54	33.46	7.53

LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

SPECIES ICRFLO/MOUSE COMPOUND 007784249

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA98 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
------	-----	----------------------	-----------------------	-----------------------	---------------------	-----------------------	-----------------------

ACT A+C 78.27 6.72 9.23 10.74 32.00 5.09 8.16

ACT A-C 68.18 10.10 6.99 7.24 31.32 8.21 4.36

ACT ALI 92.47 12.40 10.09 10.51 40.83 15.23 6.53

ACT ALU 129.25 11.26 10.80 11.33 39.13 7.66 5.92

ACT PLI 141.76 209.75 69.01 668.98 126.93 203.20 76.46

ACT PLU 114.03 13.36 16.48 18.52 42.01 52.64 8.31

ACT L11 17.90 7.34 19.82 14.38 12.23 15.68 5.64

ACT L12 33.66 16.99 17.44 10.63 15.25 9.04 5.91

ACT L13 17.82 10.53 13.79 9.81 8.42 11.30 5.47

ACT LU1 27.92 9.33 17.37 7.01 19.50 14.22 6.53

ACT LU2 23.88 7.44 14.61 9.06 11.43 13.82 6.40

ACT LU3 28.42 20.61 15.22 7.36 17.15 13.47 6.30

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM

REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

SPECIES SPRD/AW/RAT

COMPOUND 007784249

TEST	ORG	TAI00 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	NEGATIVE CONTROLS
ACT	A+C	76.96	6.17	8.43	4.62	14.02	18.42	4.59	
ACT	A-C	64.02	3.41	6.23	6.66	14.18	11.04	2.59	
ACT	ALI	76.31	6.98	8.14	11.94	11.29	19.58	5.60	
ACT	ALU	92.59	5.85	10.86	11.70	12.36	21.12	5.71	
ACT	PLI	225.23	329.92	91.38	122.04	135.13	63.49	68.82	POSITIVE CONTROLS
ACT	PLU	96.88	5.65	6.70	180.65	179.14	15.03	5.01	
ACT	L11	108.36	5.25	5.00	9.47	29.70	1.72	0.76	TEST COMPOUND
ACT	L12	44.05	6.19	7.59	9.16	28.14	9.92	4.82	
ACT	L13	88.86	5.45	8.59	5.75	28.42	5.00	5.59	
ACT	LU1	81.40	4.59	9.31	8.14	38.78	4.75	1.06	
ACT	LU2	68.33	5.71	5.73	8.43	18.52	5.77	3.12	
ACT	LU3	58.48	4.15	8.75	5.75	21.48	1.41	1.81	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

SPECIES RHECUS/MONKEY COMPOUND 007784249

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	NEGATIVE CONTROLS
ACT	A+C	66.44	9.89	7.04	18.56	4.03	14.83	4.84
ACT	A-C	52.30	7.21	4.84	15.04	2.11	10.34	2.48
ACT	ALI	34.39	8.99	8.47	20.04	6.60	15.43	12.92
ACT	ALU	35.53	5.99	7.10	18.40	3.80	18.41	8.20
ACT	PLI	81.25	149.45	26.81	201.96	270.39	36.48	56.77
ACT	PLU	34.77	10.56	30.49	17.07	5.15	16.21	5.60
								POSITIVE CONTROLS
ACT	LII	41.93	8.33	6.65	18.41	11.39	14.40	6.67
ACT	L12	38.15	7.65	8.09	15.92	10.47	11.15	5.90
ACT	LI3	49.53	8.90	9.12	10.38	5.60	14.31	5.77
ACT	LUI	44.45	9.43	5.62	15.21	6.26	12.36	6.18
ACT	LU2	46.87	10.00	7.35	16.76	5.84	12.21	7.36
ACT	LU3	33.16	6.55	8.94	15.14	2.42	12.06	7.12

DATA TABLE TERMS AND ABBREVIATIONS

<u>ABBREVIATION OR TERM</u>	<u>DEFINITION OR EXPLANATION</u>																																		
COMPOUND	Client designated compound number appears in this column.																																		
TEST CODES	<table style="margin-left: 20px; border-collapse: collapse;"> <tr><td>NAN</td><td>= Nonactivation: Solvent Control</td></tr> <tr><td>NAP</td><td>= Nonactivation: Positive Control</td></tr> <tr><td>NA1</td><td>= Nonactivation: Test Compound Dose 1</td></tr> <tr><td>NA2, etc.</td><td>= Reflects the other dose level(s)</td></tr> <tr><td> </td><td></td></tr> <tr><td>A+C</td><td>= Negative Chemical Control for ACP</td></tr> <tr><td>A-C</td><td>= Activation: Solvent Control</td></tr> <tr><td>ALI</td><td>= Activation: Homogenate Control (Liver)</td></tr> <tr><td>ALU</td><td>= Activation: Homogenate Control (Lung)</td></tr> <tr><td>ACP</td><td>= Activation: Positive Control</td></tr> <tr><td>ACT</td><td>= Activation Test</td></tr> <tr><td> </td><td></td></tr> <tr><td>LI</td><td>= Liver Tissue Activation Fraction</td></tr> <tr><td>LU</td><td>= Lung Tissue Activation Fraction</td></tr> <tr><td>KI</td><td>= Kidney Tissue Activation Fraction</td></tr> <tr><td>TE</td><td>= Testes Tissue Activation Fraction</td></tr> <tr><td>1,2, etc.</td><td>= Dose Levels</td></tr> </table>	NAN	= Nonactivation: Solvent Control	NAP	= Nonactivation: Positive Control	NA1	= Nonactivation: Test Compound Dose 1	NA2, etc.	= Reflects the other dose level(s)	 		A+C	= Negative Chemical Control for ACP	A-C	= Activation: Solvent Control	ALI	= Activation: Homogenate Control (Liver)	ALU	= Activation: Homogenate Control (Lung)	ACP	= Activation: Positive Control	ACT	= Activation Test	 		LI	= Liver Tissue Activation Fraction	LU	= Lung Tissue Activation Fraction	KI	= Kidney Tissue Activation Fraction	TE	= Testes Tissue Activation Fraction	1,2, etc.	= Dose Levels
NAN	= Nonactivation: Solvent Control																																		
NAP	= Nonactivation: Positive Control																																		
NA1	= Nonactivation: Test Compound Dose 1																																		
NA2, etc.	= Reflects the other dose level(s)																																		
A+C	= Negative Chemical Control for ACP																																		
A-C	= Activation: Solvent Control																																		
ALI	= Activation: Homogenate Control (Liver)																																		
ALU	= Activation: Homogenate Control (Lung)																																		
ACP	= Activation: Positive Control																																		
ACT	= Activation Test																																		
LI	= Liver Tissue Activation Fraction																																		
LU	= Lung Tissue Activation Fraction																																		
KI	= Kidney Tissue Activation Fraction																																		
TE	= Testes Tissue Activation Fraction																																		
1,2, etc.	= Dose Levels																																		
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration																																		
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = $\times 10^6$).																																		
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.																																		
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.																																		
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.																																		
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.																																		
CONTAM	Presence of contamination on any plates.																																		

DATA TABLE TERMS AND ABBREVIATIONS (continued)

<u>ABBREVIATION OR TERM</u>	<u>DEFINITION OR EXPLANATION</u>
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (<u>Macaca mulatta</u>)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan



BIONETICS

Litton

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 75-91, Aluminum Potassium Sulfate, was evaluated for genetic activity in a series of in vitro microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

The test compound, FDA 75-91, Aluminum Potassium Sulfate, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

DL. Jayannath 7-28-77

Rev David J. Brusick, Ph.D. Date
Director
Department of Molecular
Toxicology

Reviewed by:

Robert J. Weir 7-28-77
Robert J. Weir, Ph.D. Date
Vice President



BIONETICS

VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.



BIONETICS

Litton

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.



BIONETICS
Litton

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the his⁻ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.



BIONETICS

Litton

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

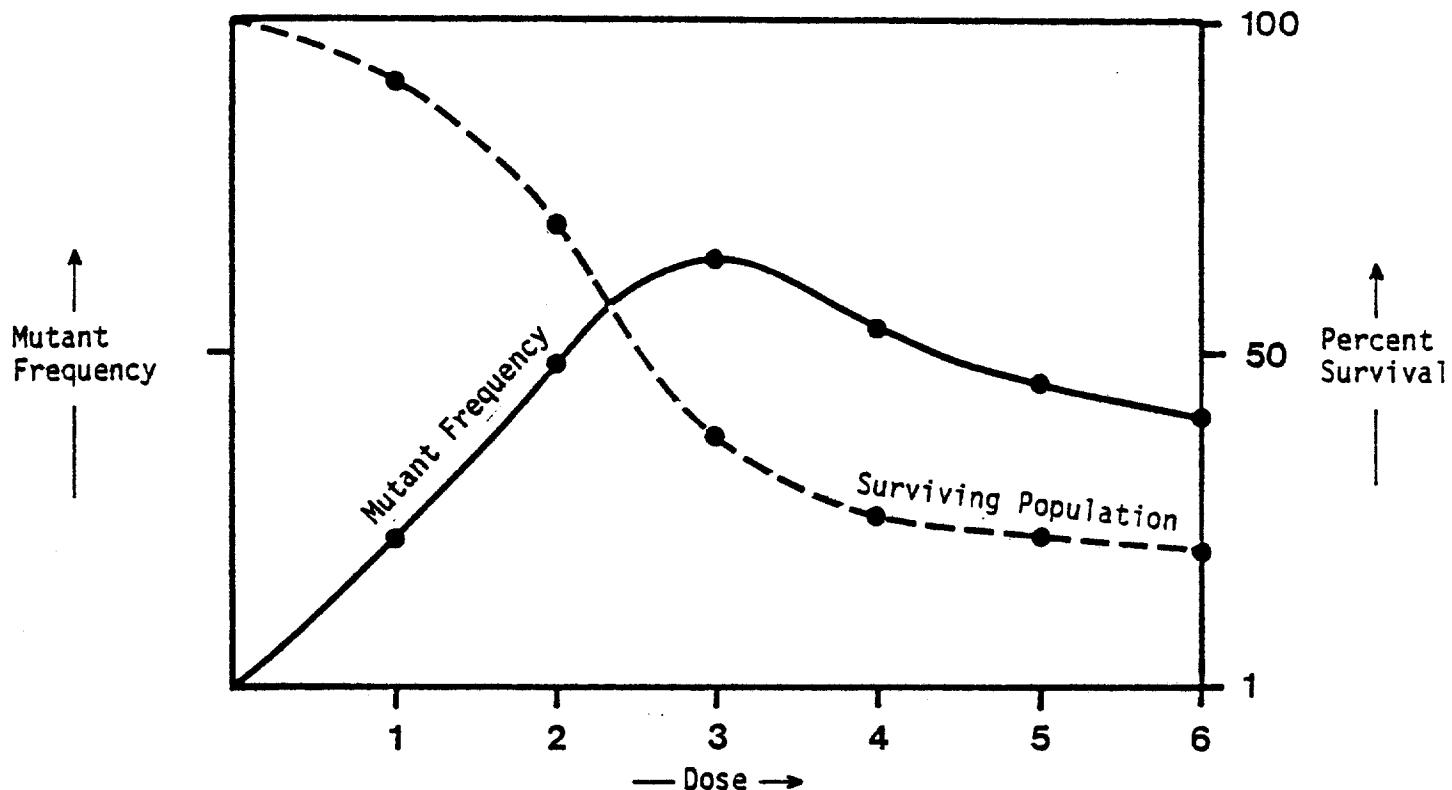
The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.



BIONETICS

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT

- (1) Dose levels 1, 2 & 3 were used
- (2) Dose levels 2, 3 & 4 were used
- (3) Dose levels 3, 4 & 5 were used

OBSERVED DOSE RESPONSE

- A typical positive dose response set of data would be obtained.
- The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
- Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX
Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST	ORG ID	DETECTOR	TA100 CONCENTRATION	SPECIES	PROJECT /	2672	DATE - 07/22/77
706746	223-76-2102				POP0 EP+6	MUT1 EP+0	FREQ1 EP-8		
NAN		NAN	SOLVENT		0445	1505		338.20	0
NAP		NAP	EMS 0.066%		0773	4487		580.47	0
0077H4249		NA1	0002-0 PCT.		0517	1014		196.13	0
0077H4249		NA2	0001-0 PCT.		0635	1556		186.35	0
0077H4249		NA3	0005-1 PCT.		0813	1670		205.41	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 706755			CONTRACT 223-76-2102	DETECTOR TA1535	SPECIES /	PROJECT 2672	DATE - 07/22/17
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUTL	FREQ1	CONTAM
				EP+6	EP+0	EP-B	
NAN		SOLVENT		0385	0049	12.73	0
NAP		EMS 0.2%		0586	0834	142.32	0
007784249	NA1	0002-0 PCT.		0210	0036	17.14	0
007784249	NA2	0001-0 PCT.		0292	0048	16.44	0
007784249	NA3	0005-1 PCT.		0310	0046	14.84	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	DETECTOR	SPECIES	PROJECT	DATE
706651	223-76-2102	TAI537	/	2672	- 07/22/77
COMPOUND	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1
NAN	SOLVENT	EP+6	EP+0	EP-8	CONTAM
NAP	QM 13 UG/ML	0.341	0.340	99.71	0
007784249 NA1	0002-0 PCT.	2024	0155	7.66	0
007784249 NA2	0001-0 PCT.	1739	0160	9.20	0
007784249 NA3	0005-1 PCT.	1044	0116	11.11	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 709652			CONTRACT 223-76-2102	DETECTOR TA1538	SPECIES /	PROJECT 2672	DATE - 07/22/77
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1 EP+6 EP+0	FREQ1 EP-B	CONTAM
NAN		SOLVENT		0405	0048	11.85	0
NAP	NF 667	UG/ML		0405	0486	120.00	0
007784249	NA1	0002-0 PCT.		0454	0018	3.96	1
007784249	NA2	0001-0 PCT.		0441	0011	2.49	0
007784249	NA3	0005-1 PCT.		0422	0025	5.92	1

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 706757			CONTRACT 223-76-2102 DETECTOR TA98		SPECIES	PROJECT /	DATE - 07/22/77	
COMPOUND	TEST	ORG	10	CONCENTRATION	POPU	MUT1 EP+6 EP+0	FREQ1 EP-8	CONTAM
NAN		SOLVENT			0.306	0.030	9.80	0
NAP		NF	667	UG/ML	0876	1710	195.21	0
007784249	NA1		0002-0	PCT.	0.453	0.045	9.93	0
007784249	NA2		0001-0	PCT.	0574	0.044	7.67	0
007784249	NA3		0005-1	PCT.	0686	0.038	5.54	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 711055			CONTRACT 223-76-2102		PROJECT /		PROJECT 2672		DATE - 07/22/77		
COMPOUND	TEST	ORG ID	DETECTOR	000004	SPECIES		MUT1 EP+4	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
NAN		SOLVENT			1178	0198	0161	16.81	13.67	0	
NAP		EMS 1.0 %			0260	0562	0493	216.15	189.62	0	
007784249	NA1	'0005-0 PCT.			2252	0431	0117	19.14	5.20	0	
007784249	NA2	0025-1 PCT.			0979	0088	0080	8.99	8.17	0	
007784249	NA3	0125-2 PCT.			0810	0271	0061	33.46	7.53	0	

REPORT EXR33 LITTON ALIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 708852			CONTRACT 223-76-2102	DETECTOR TA100	SPECIES ICRFLO/MOUSE	PROJECT 2672	DATE - 07/22/77
COMPOUND	TEST ID	ORG	TEST ID	CONCENTRATION	POPNU	MUT1	FREQ1
					EP+6	EP+0	EP+8
A+C		DHN	90 UM/ML	0833	0652	78.27	0
A-C		SOLVENT		0682	0465	68.18	0
ALI		TISSUE		0810	0749	92.47	0
ALU		TISSUE		0530	0685	129.25	0
ACP	L1	DHN	90 UM/ML	0546	0774	141.76	0
ACP	LU	DHN	90 UM/ML	0556	0634	114.03	0
007784249	ACT	L11	0002-0 PCT.	1106	0198	17.90	0
007784249	ACT	L12	0001-0 PCT.	0621	0209	33.66	0
007784249	ACT	L13	0005-1 PCT.	0937	0167	17.82	0
007784249	ACT	LU1	0002-0 PCT.	0437	0122	27.92	0
007784249	ACT	LU2	0001-0 PCT.	0649	0155	23.88	0
007784249	ACT	LU3	0005-1 PCT.	0753	0214	28.42	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	223-76-2102	DETECTOR	TA1535	SPECIES	ICRFLO/MOUSE	PROJECT	2672	DATE	- 07/22/77
COMPOUND	TEST	OHG	ID	CONCENTRATION	POPU	MUT1	FREQ1			
A+C		DMN	90	UM/ML	0625	0042	EP+6	EP-8		
A-C		SOLVENT			0515	0052			6.72	0
ALI		TISSUE			0492	0061			10.10	0
ALU		TISSUE			0506	0057			12.40	0
ACP	L1	DMN	90	UM/ML	0318	0667			11.26	0
ACP	LU	DMN	90	UM/ML	0217	0029			209.75	0
007784249	ACT	L11	0002-0	PCT.	0504	0037			13.36	0
007784249	ACT	L12	0001-0	PCT.	0306	0052			7.34	0
007784249	ACT	L13	0005-1	PCT.	0475	0050			16.99	0
007784249	ACT	L14	0002-0	PCT.	0343	0032			10.53	0
007784249	ACT	LU2	0001-0	PCT.	0363	0027			9.33	0
007784249	ACT	LU3	0005-1	PCT.	0330	0068			7.44	0
									20.61	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST	ORG ID	CONCENTRATION	POPULATION	MUT1	FREQ1	DATE -
705951	223-76-2102	DETECTION	TAI537	SPECIES	ICRFLO/MOUSE	EP+6	EP-8	07/22/11
A+C	AMQ	333 UG/ML		1224	0113	9.23	0	
A-C	SOLVENT			1130	0079	6.99	0	
ALI	TISSUE			0872	0088	10.09	0	
ALU	TISSUE			0722	0078	10.80	0	
ACP	LI	AMQ 333 UG/ML		1097	0757	69.01	0	
ACP	LU	AMQ 333 UG/ML		0801	0148	18.48	0	
007784249	ACT	L11	0002-0 PCT.	0454	0090	19.82	0	
007784249	ACT	L12	0001-0 PCT.	0516	0090	17.44	0	
007784249	ACT	L13	0005-1 PCT.	0464	0064	13.79	0	
007784249	ACT	LW1	0002-0 PCT.	0495	0086	17.37	2	
007784249	ACT	LW2	0001-0 PCT.	0568	0083	14.61	0	
007784249	ACT	LW3	0005-1 PCT.	0624	0095	15.22	0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 708853			CONTRACT 223-76-2102	DETECTOR TA1538	SPECIES ICRFLO/MOUSE	PROJECT 2672	DATE - 07/22/77
COMPOUND	TEST ID	OKG	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
				EP+6	EP+0	EP-B	
A+C			ANTH 67 UG/ML	0633	0068	10.74	0
A-C			SOLVENT	0898	0065	7.24	0
ALI			TISSUE	0590	0062	10.51	2
ALU			TISSUE	0547	0062	11.33	2
ACP	LI	ANTH 67 UG/ML		0332	2221	668.98	0
ACP	LU	ANTH 67 UG/ML		0583	0108	18.52	2
007784249	ACT	LI1	0002-0 PCT.	0619	0089	14.38	2
007784249	ACT	LI2	0001-0 PCT.	0715	0076	10.63	2
007784249	ACT	LI3	0005-1 PCT.	0744	0073	9.81	2
007784249	ACT	LU1	0002-0 PCT.	0585	0041	7.01	2
007784249	ACT	LU2	0001-0 PCT.	0519	0047	9.06	2
007784249	ACT	LU3	0005-1 PCT.	0516	0038	7.36	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 706951			CONTRACT 223-76-2102 DETECTOR TA98		SPECIES ICRFLO/MOUSE		PROJECT 2672	DATE - 07/22/77
COMPOUND	TEST	ORG ID	CONCENTRATION	POPNU	MUT1	FREQ1		CONTAM
A+C			ANTH 67 UG/ML	13222	0423	32.00		0
A-C		SOLVENT		1306	0409	31.32		0
ALI		TISSUE		1298	0530	40.83		2
ALU		TISSUE		1086	0425	39.13		0
ACP	LI	ANTH 67 UG/ML	1051	1334	126.93			0
ACP	LU	ANTH 67 UG/ML		0845	0355	42.01		0
007784249	ACT	LI1	0002-0 PCT.	1128	0138	12.23		0
007784249	ACT	LI2	0001-0 PCT.	0859	0131	15.25		0
007784249	ACT	LI3	0005-1 PCT.	1342	0113	8.42		0
007784249	ACT	LU1	0002-0 PCT.	0636	0124	19.50		2
007784249	ACT	LU2	0001-0 PCT.	1295	0148	11.43		1
007784249	ACT	LU3	0005-1 PCT.	0939	0161	17.15		0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 710953			CONTRACT 223-76-2102 DETECTOR 0000D4		SPECIES ICARFLO/MOUSE		PROJECT 2672		DATE - 07/22/77	
COMPOUND	TEST	ORG ID	CONCENTRATION	POP	MUT1	MUT2	FREQ1	FREQ2		CONTAM
				EP+4	EP+1	EP+1	EP-5	EP-5		
A+C		DMN 90 UM/ML		1789	0091	0146	5.09	8.16	0	
A-C	SOLVENT			1193	0098	0052	8.21	4.36	0	
ALI	TISSUE			1333	0203	0087	15.23	6.53	0	
ALU	TISSUE			1605	0123	0095	7.66	5.92	0	
ACP	L1	DMN 90 UM/ML		1627	3306	1244	203.20	76.46	0	
ACP	LU	DMN 90 UM/ML		1721	0906	0143	52.64	8.31	0	
007784249	ACT	L11	0005-0 PCT.	1454	0228	0082	15.68	5.64	0	
007784249	ACT	L12	0025-1 PCT.	1726	0156	0102	9.04	5.91	0	
007784249	ACT	L13	0125-2 PCT.	1372	0155	0075	11.30	5.47	0	
007784249	ACT	LU1	0005-0 PCT.	1638	0233	0107	14.22	6.53	0	
007784249	ACT	LU2	0025-1 PCT.	1187	0164	0076	13.82	6.40	0	
007784249	ACT	LU3	0125-2 PCT.	1477	0199	0093	13.47	6.30	0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102				PROJECT 2672		DATE - 07/22/77
EXPERIMENT 708954		DEFECTOR TA100		SPECIES SPRDAN/RAT		
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1
				EP+6	EP+0	EP-B
A+C		DMN 90 UM/ML		0638	0491	76.96
A-C	SOLVENT			0681	0436	64.02
ALI	TISSUE			0688	0525	76.31
ALU	TISSUE			0459	0425	92.59
ACP	LI	DMN 90 UM/ML		0218	0491	225.23
ACP	LU	DMN 90 UM/ML		0609	0590	96.88
007784249	ACT	LI1 0002-0 PCT.		0586	0635	108.36
007784249	ACT	LI2 0001-0 PCT.		1269	0559	44.05
007784249	ACT	LI3 0005-1 PCT.		0781	0694	88.86
007784249	ACT	LU1 0002-0 PCT.		0860	0700	81.40
007784249	ACT	LU2 0001-0 PCT.		0963	0658	68.33
007784249	ACT	LU3 0005-1 PCT.		0961	0562	58.48

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	DETECTOR	TA1535	SPECIES	SPRDAM/RAT	PROJECT	2672	DATE - 07/22/77
708851	223-76-2102			POPU	MUT1	FREQ1		
COMPOUND	TEST	ID	CONCENTRATION	EP+6	EP+0	EP-8		CONTAM
A+C		DHN 90 UM/ML	0.664	0.041		6.17		0
A-C		SOLVENT	0.822	0.028		3.41		0
ALI		TISSUE	0.802	0.056		6.98		0
ALU		TISSUE	0.598	0.035		5.85		0
ACP	LI	DHN 90 UM/ML	0.264	0.071		329.92		0
ACP	LU	DHN 90 UM/ML	0.797	0.045		5.65		0
007784249	ACT	LI1	0002-0 PCT.	0.362	0.019	5.25		0
007784249	ACT	LI2	0001-0 PCT.	0.452	0.028	6.19		0
007784249	ACT	LI3	0005-1 PCT.	0.495	0.027	5.45		0
007784249	ACT	LU1	0002-0 PCT.	0.392	0.018	4.59		0
007784249	ACT	LU2	0001-0 PCT.	0.543	0.031	5.71		0
007784249	ACT	LU3	0005-1 PCT.	0.844	0.035	4.15		0

REPORT EXR33 LITTON RIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	DETECTOR	TAI537	SPECIES	SPREAD/RAT	PROJECT	2672	DATE	- 07/22/77
COMPOUND	TEST	ID	CONCENTRATION	POPU	MUT1	FREQ1	EP-8	CONTAM	
			AMQ 333 UG/ML	EP+0	EP+0				
A+C			AMQ 333 UG/ML	1341	0113	8.43		0	
A-C		SOLVENT		1284	0080	6.23		0	
AL I		TISSUE		0799	0065	8.14		0	
ALU		TISSUE		0746	0081	10.86		0	
ACP	L I	AMQ 333 UG/ML		0612	0742	91.38		0	
ACP	LU	AMQ 333 UG/ML		2104	0141	6.70		0	
007784249	ACT	LI 1	0002-0 PCT.	1420	0071	5.00		2	
007784249	ACT	LI 2	0001-0 PCT.	0948	0072	7.59		0	
007784249	ACT	LI 3	0005-1 PCT.	0908	0078	8.59		0	
007784249	ACT	LU 1	0002-0 PCT.	1149	0107	9.31		0	
007784249	ACT	LU 2	0001-0 PCT.	1501	0086	5.73		0	
007784249	ACT	LU 3	0005-1 PCT.	1189	0104	8.75		0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 712552		CONTRACT 223-76-2102		PROJECT 2672		DATE - 07/22/77		
		DETECTOR TA1538		SPECIES SPRDAW/RAT				
COMPOUND	ORG ID	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	
					EP+6	EP+0	EP-8	
							CONTAM	
A+C			ANTH 67	UG/ML	1429	0.066	4.62	0
A-C			SOLVENT		1217	0.081	6.66	0
ALI			TISSUE		0896	0.107	11.94	0
ALU			TISSUE		0684	0.080	11.70	0
ACP	LI	ANTH 67	UG/ML		1366	1.667	122.04	0
ACP	LU	ANTH 67	UG/ML		0713	1.288	180.65	0
007784249	ACT	LI1	0002-0	PCT.	0971	0.092	9.47	0
007784249	ACT	LI2	0001-0	PCT.	1190	0.109	9.16	0
007784249	ACT	LI3	0005-1	PCT.	1375	0.079	5.75	0
007784249	ACT	LU1	0002-0	PCT.	0700	0.057	8.14	0
007784249	ACT	LU2	0001-0	PCT.	0842	0.071	8.43	0
007784249	ACT	LU3	0005-1	PCT.	1078	0.062	5.75	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST	ORG ID	ORG CONCENTRATION	SPECIES	SPRDW/RAT	PROJECT	DATE -
708951	223-76-2102	DETECTOR TA98			MUT1	SPRDW	2672	07/22/77
				EP+6	EP+0		FREQ1	
A+C	ANTH 67 ug/ml			0906	0127		EP+8	
A-C	SOLVENT			0931	0132		14.02	0
ALI	TISSUE			0912	0103		14.18	0
ALU	TISSUE			0793	0098		11.29	0
ACP	LI ANTH 67 ug/ml			0874	1181		12.36	0
ACP	LU ANTH 67 ug/ml			0489	0876		135.13	0
ACP	LU ANTH 67 ug/ml			0489	0876		179.14	0
007784249	ACT LI1 0002-0 PCT.			0303	0090		29.70	0
007784249	ACT LI2 0001-0 PCT.			0462	0130		28.14	0
007784249	ACT LI3 0005-1 PCT.			0563	0160		28.42	0
007784249	ACT LU1 0002-0 PCT.			0361	0140		38.78	0
007784249	ACT LU2 0001-0 PCT.			0826	0153		18.52	0
007784249	ACT LU3 0005-1 PCT.			0633	0136		21.48	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	710844	CONTRACT	223-76-2102	DETECTOR	0000D4	SPECIES	SPRDW/RAT	PROJECT	2672	DATE	- 07/22/77
COMPOUND	TEST ID	ORG	CONCENTRATION		POPU	MUT1	MUT2	FREQ1	FREQ2		
				EP+4	EP+1	EP+1	EP+5	EP+5	EP+5		CONTAM
A+C		DMN	90 UM/ML		1851	0341	0085	18.42	4.59		0
A-C	SOLVENT				1776	0196	0046	11.04	2.59		0
ALI	TISSUE				1767	0346	0099	19.58	5.60		0
ALU	TISSUE				1733	0366	0099	21.12	5.71		0
ACP	LI	DMN	90 UM/ML		1690	1073	1163	63.49	68.82		0
ACP	LU	DMN	90 UM/ML		1876	0282	0094	15.03	5.01		0
007784249	ACT	L11	0005-0	PCT.	1454	0025	0011	1.72	0.76		0
007784249	ACT	L12	0025-1	PCT.	1845	0183	0089	9.92	4.82		0
007784249	ACT	L13	0125-2	PCT.	1699	0085	0095	5.00	5.59		0
007784249	ACT	L01	0005-0	PCT.	1895	0090	0020	4.75	1.06		0
007784249	ACT	LU2	0025-1	PCT.	1698	0098	0053	5.77	3.12		0
007784249	ACT	LU3	0125-2	PCT.	1708	0024	0031	1.41	1.81		0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 711851		CONTRACT 223-76-2102		DETECTOR TAI00		SPECIES Rhesus/Monkey		PROJECT 2672		DATE - 07/22/77	
COMPOUND	TEST ID	OHG	ID	CONCENTRATION		POPU	MUTI	REQ1		CONTAM	
A+C		DMN	90	UM/ML		0432	0287	66.44		0	
A-C	SOLVENT					0543	0284	52.30		0	
ALI	TISSUE					1742	0599	34.39		0	
ALU	TISSUE					1489	0529	35.53		0	
ACP	LI	DMN	90	UM/ML		0896	0728	81.25		0	
ACP	LU	DMN	90	UM/ML		1930	0671	34.77		0	
007784249	ACT	LI1	0002-0	PCT.		1388	0582	41.93		0	
007784249	ACT	LI2	0001-0	PCT.		1929	0736	38.15		0	
007784249	ACT	LI3	0005-1	PCT.		1391	0689	49.53		0	
007784249	ACT	LU1	0002-0	PCT.		1298	0577	44.45		0	
007784249	ACT	LU2	0001-0	PCT.		1231	0577	46.87		0	
007784249	ACT	LU3	0005-1	PCT.		1725	0572	33.16		0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST	ORG ID	CONCENTRATION	POPUL	MUT1	FREQ1	PROJECT	DATE -
706853	223-76-2102	DETECTOR TA1535		EP+6	EP+0	EP+8		2672	07/22/77
COMPOUND							CONTAM		
A+C		DMN	90 UM/ML	0617	0061	9.89			0
A-C		SOLVENT		0499	0036	7.21			0
ALI		TISSUE		0845	0076	8.99			0
ALU		TISSUE		0784	0047	5.99			2
ACP	L1	DMN	90 UM/ML	0364	0544	149.45			0
ACP	LU	DMN	90 UM/ML	0824	0087	10.56			0
007784249	ACT	L11	0002-0 PCT.	0624	0052	8.33			0
007784249	ACT	L12	0001-0 PCT.	0614	0047	7.65			0
007784249	ACT	L13	0005-1 PCT.	0483	0043	8.90			0
007784249	ACT	LU1	0002-0 PCT.	0541	0051	9.43			0
007784249	ACT	LU2	0001-0 PCT.	0510	0051	10.00			0
007784249	ACT	LU3	0005-1 PCT.	0870	0057	6.55			0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 710352			CONTRACT 223-76-2102 DETECTOR TA1537		SPECIES RHESUS/MONKEY		PROJECT 2672		DATE - 07/22/77	
COMPOND	ORG	ID	TEST	CONCENTRATION	POPU	MUT1	FREQ1	EP+0	EP+8	CONTAM
A+C	AMQ	333 UG/ML		1406	0.099		7.04			0
A-C	SOLVENT			1365	0.066		4.84			0
ALI	TISSUE			1700	0.144		8.47			0
ALU	TISSUE			1000	0.071		7.10			0
ACP	LI	AMQ 333 UG/ML		1035	0.492		26.81			0
ACP	LU	AMQ 333 UG/ML		0797	0.243		30.49			0
007784249	ACT	L11	0002-0 PCT.	0932	0.062		6.65			0
007784249	ACT	L12	0001-0 PCT.	0643	0.052		8.09			0
007784249	ACT	L13	0005-1 PCT.	0833	0.076		9.12			0
007784249	ACT	LU1	0002-0 PCT.	1139	0.064		5.62			0
007784249	ACT	LU2	0001-0 PCT.	0544	0.040		7.35			0
007784249	ACT	LU3	0005-1 PCT.	0727	0.065		8.94			0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 711151			CONTRACT 223-76-2102	DETECTOR TA1530	SPECIES RHESUS/MONKEY	PROJECT 2672	DATE - 07/22/77
COMPOUND	TEST ID	ORG	CONCENTRATION	POPU	MUTI	FREQ1	CONTAM
A+C		ANTH 67	UG/ML	0555	0103	18.56	0
A-C		SOLVENT		0565	0085	15.04	3
ALI		TISSUE		0554	0111	20.04	0
ALU		TISSUE		0614	0113	18.40	0
ACP	LI	ANTH 67	UG/ML	0561	1133	201.96	2
ACP	LU	ANTH 67	UG/ML	0662	0113	17.07	0
007784249	ACT	LI1	0002-0 PCT.	0440	0081	18.41	1
007784249	ACT	LI2	0001-0 PCT.	0628	0100	15.92	0
007784249	ACT	LI3	0005-1 PCT.	0742	0077	10.38	0
007784249	ACT	LU1	0002-0 PCT.	0697	0106	15.21	0
007784249	ACT	LU2	0001-0 PCT.	0572	0096	16.78	0
007784249	ACT	LU3	0005-1 PCT.	0634	0096	15.14	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 708953			CONTRACT 223-76-2102	DETECTOR TA98	SPECIES RHECUS/MONKEY	PROJECT 2672	DATE - 07/22/77
COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
A+C			ANTH 67 UG/ML	0596	0024	4.03	0
A-C	SOLVENT			0758	0016	2.11	0
ALI	TISSUE			0863	0057	6.60	0
ALU	TISSUE			0868	0033	3.80	0
ACP	LI	ANTH 67 UG/ML		0760	2055	270.39	0
ACP	LU	ANTH 67 UG/ML		0815	0042	5.15	0
007784249	ACT	L11	0002-0 PCT.	0202	0023	11.39	0
007784249	ACT	L12	0001-0 PCT.	0277	0029	10.47	0
007784249	ACT	L13	0005-1 PCT.	0393	0022	5.60	0
007784249	ACT	LU1	0002-0 PCT.	0479	0030	6.26	0
007784249	ACT	LU2	0001-0 PCT.	0394	0023	5.84	0
007784249	ACT	LU3	0005-1 PCT.	0660	0016	2.42	0

REPORT EXP33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
EXPERIMENT 715954 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102				PROJECT 2672				DATE - 07/22/77	
EXPERIMENT 715954 DETECTOR 0000D4				SPECIES Rhesus/Monkey					
COMPOUND	TEST ID	ORG	CONCENTRATION	POPUP	MUT1	MUT2	FREQ1	FREQ2	CONTAM
			EP+4	EP+1	EP+1	EP+5	EP+5	EP+5	
A+C		DMN	90 UM/ML	1983	0294	0096	14.83	4.84	0
A-C	SOLVENT			1451	0150	0036	10.34	2.48	0
ALI	TISSUE			1231	0190	0159	15.43	12.92	0
ALU	TISSUE			1537	0283	0126	18.41	8.20	0
ACP	LI	DMN	90 UM/ML	2163	0789	1228	36.48	56.77	4
ACP	LU	DMN	90 UM/ML	1999	0324	0112	16.21	5.60	0
007784249	ACT	L11	0005-0 PCT.	1979	0285	0132	14.40	6.67	0
007784249	ACT	L12	0025-1 PCT.	2018	0225	0119	11.15	5.90	0
007784249	ACT	L13	0125-2 PCT.	1908	0273	0110	14.31	5.77	0
007784249	ACT	L01	0005-0 PCT.	2152	0266	0133	12.36	6.18	0
007784249	ACT	L02	0025-1 PCT.	2064	0252	0152	12.21	7.36	0
007784249	ACT	L03	0125-2 PCT.	2064	0249	0147	12.06	7.12	0